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Filaggrin loss of function mutations are a factor in patients with multiple contact allergies.

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Conflicts of Interest. W. H. I. McLean has filed patents on genetic testing and therapy development aimed at the filaggrin gene. The rest of the authors have declared that they have no conflict of interest.

Summary.

Background

Polysensitivity is defined as 3 or more positive patch test reactions. The role of *Filaggrin* loss of function mutations in patients with polysensitivity has not been studied when barrier bypass and possible preceding barrier damage was excluded.

Methods

169 Patients with 3 or more, non-cross reacting, positive patch test reactions were prospectively enrolled in this study. Exclusion criteria were a history of hand dermatitis, nickel and metal implants and stasis dermatitis. Subjects were patch tested to the North American extended patch test series as well as relevant other haptens. DNA was obtained and sequenced for the following *Filaggrin* (*FLG*) loss of function mutations (R501X, 2282del4, R2447X and S3247X).

Results

169 patients were genotyped for the four *FLG* mutations. There is a significant association between R501X mutations and polysensitivity. For the other 3 tested mutations, there was no significant association with polysensitivity. When all mutations are combined, there was a significant association between loss of function *FLG* mutations and polysensitivity in patients with a history of atopic dermatitis.

Conclusion

When skin barrier bypass is excluded there is a significant association between polysensitivity and *FLG* loss of function mutations.

Keywords. Polysensitivity, contact dermatitis, skin barrier, *Filaggrin,* loss of function mutations

1. Introduction

Polysensitivity has been defined as the three or more positive patch tests.[1] Patients with atopic dermatitis or other forms of eczema are more likely to develop polysensitivity. This suggests that defects in barrier function as caused by null mutations in *Filaggrin (FLG)* or other induced defects in barrier function may play a predisposing role. Patients with chronic leg ulcers are also prone to polysensitivity.[2,3] In a study involving 163 individuals with polysensitivity 13 were found to have null mutations in *FLG* (R501X or 2282del4) but this finding was not significant.[4] The role of loss of function mutations in *FLG* in contact dermatitis is controversial. Most studies examining this have focused on this relationship in hand dermatitis with conflicting results.[4-6] Barrier dysfunction from gene mutations, besides *FLG*, have also been described. Molin et al reported an association between allergic contact dermatitis on the hands and combined deletions in genes encoding late cornified envelope-3 (*LCE3B and LCE3C*). [7]

In this Canadian study, we evaluated the predisposing role of *FLG* loss of functions mutations in polysensitivity. We have attempted to exclude any subjects who would have a high likelihood of confounding owing to sensitization through skin barrier bypass; this includes prior hand dermatitis, where damage to the skin barrier could allow easier penetration and so promote the development of multiple sensitivities.

2. Methods

Patients (n=169) were recruited prospectively (2009 to 2017) from 4 Canadian University based patch test clinics (Universities of Saskatchewan [PRH] and Ottawa [MP], Ontario, McGill University [DS], Quebec and Dalhousie University [PRH]), Nova Scotia. Saskatchewan is in Western Canada and Nova Scotia is in the East. Ottawa and Montreal are situated geographically in closer proximity. The provinces of Nova Scotia and Saskatchewan have populations of only 1 million while the cities of Ottawa and Montreal have populations over one million each.

All patients were patch tested on day 0 (D0) to the North American extended series of at least 65 haptens or with the North American Contact Dermatitis Group series of 70 allergens along with additional testing depending on the clinical presentations. The patches were removed on D2 and read again on day D4 or D5. For some haptens the result was again read between D5 and D10. Patch test material was acquired from Chemotechnique Diagnostics (Vellinge, Sweden) or from AllergEAZE (SmartPractice, Calgary, Canada) and applied using either Finn Chambers (SmartPractice) or IQ Ultra (Chemotechnique Diagnostics) and Scanpore tape (Norgesplaster, Vennesla, Norway). Methods for patch testing, evaluation of reactions and data recording followed the North American Contact Dermatitis Group protocol.[8,9]

Patients with 3 or more positive patch tests were included in this study. Haptens known to cross-react such as formaldehyde and formaldehyde releases were counted as a single positive for the purpose of inclusion. In order to exclude skin barrier bypass from piercings, positive reaction to nickel was not counted as one of the 3 positive reactions and patients with metal implants were excluded. As irritant contact dermatitis and occupational related dermatitis mostly involve hand dermatitis, we excluded patients with a prior history of hand dermatitis. In addition, patients with a history of stasis dermatitis, with and without ulceration, were excluded.

Genomic DNA was extracted from blood or saliva samples and genotyped for the following prevalent European *FLG* null mutations: R501X, 2282del4, R2447X and S3247X. *FLG* genotyping was performed by a core facility (DNA Sequencing and Services, University of Dundee, Dundee, United Kingdom) as well as at Dalhousie University. As the prevalence of these mutations is not known for other ethnic groups in Canada, the study was confined to those with self-identified European ancestry. The control population (n=891) were adult caucasian volunteers from Toronto, Ontario, whose DNA is commercially available (The Centre for Applied Genomics, Ontario Population Genomics Platform [OPGP], Toronto, Ontario, Canada). The control population was previously used in genetic studies of *FLG* loss of function mutations and genotyped for the same *FLG* mutations.[10] The age of this cohort ranged from 23 to 77 with 68.5% being female. As this is a general

population sample, the presence or absence of coexisting atopic dermatitis is not known. This control population has not been patch tested.

The association between *FLG* loss of function mutations and polysensitivity was performed using X^2-test and logistic regression analysis. A *P*-value of ≤ 0.05 was considered significant. All statistical analyses were performed using Stata Statistical Software: Release 15. College Station, Texas and SAS STAT 14.3 version 9.4 (SAS Institute, Cary, North Carolina). Institutional approval from the ethics review boards was obtained for each of the institutions directly involved with patient recruitment and informed consent was obtained from each of the participants.

3. RESULTS

A total of 169 patients met the inclusion and exclusion criteria, respectively, and were enrolled in this study. Four patients were subsequently excluded due to DNA low quality. 60 patients were recruited in Saskatchewan, 43 from Quebec, 42 from Ontario, and 16 from Nova Scotia. The mean age was 52 years with an age range from 10 to 89 years with only 4 patients below the age of 20 years. The majority of the patients was female (72%). About one third reported a history considered by the dermatologist as being consistent with atopic dermatitis, being a history of eczema starting in childhood and with a predominant flexural distribution. A self-reported family history of atopic dermatitis was recorded in 62 patients (37%). Asthma was reported in 37 patients (22%), hay fever in 64 patients (38%) and food allergies in 30 patients (18%) (Table 1).

FLG genotypes were obtained for all 165 patients. Null mutations were identified in 28 patients (17%) (Table 2). Single *FLG* mutations (heterozygous) were found in 24 patients (15%). Two patients were homozygous (one for R501X and the second for 2284del4). Two patients had 2 different mutations (compound heterozygous) one with the genotype $R501X^{+/-}$ / $2284del4^{+/-}$, and the other with $R501X^{+/-}$ / $R2247X^{+/-}$. Patients homozygous or compound heterozygous for *FLG* mutations were more likely to be younger, to have a personal history of atopic dermatitis, asthma, and food allergies, than those with heterozygous mutations or wild type *FLG* (Table 1).

They are also more likely to have a family history of asthma but not atopic dermatitis. The face and neck were more commonly affected by dermatitis in the mutant *FLG* genotype (Table 1).

There was a significant association between R501X mutations and polysensitivity with an R501X mutation in 8.5% (N=14) of patients and 4% (N=34) in the controls (X^2 test: $P = 0.008$). The odds of having a R501X mutations in the patients was 2.33 times higher than in the controls, Odds Ratio of 2.33 (95% CI: 1.13 to 4.59). For the other 3 tested mutations (2282del4, R2447X and S3247X) there was no significant association with polysensitivity. When all mutations are combined, a significant association with polysensitivity persists with 28 (15%) of the patients having a mutation compared to 11% in the controls (X^2 *P*-value=0.030). The odds ratio of the combined mutations was 1.65 (95% CI 1.05 to 2.61) for the patients compared to the controls. The proportion of *FLG* mutations increased with the number if positive patch tests (Table 3).

The 20 commonest reacting haptens are shown in Table 4. There were no significant differences in odds ratios when those with *FLG* loss of function mutations are compared to those without mutations. Owing to low counts there was insufficient power to perform multivariate analysis to adjust for atopic dermatitis and gender.

4. Discussion

In order to elicit cell-mediated hypersensitivity in allergic contact dermatitis, the allergen/hapten must penetrate the epidermal barrier constituted by both the fully keratinized stratum corneum matrix as well as the scaffold proteins of tight junctions.[11] Damage to the barrier either by a prior irritant dermatitis or eczema enhances absorption and facilitates an allergic response. Whether genetic modifications in the skin barrier predispose to allergic contact dermatitis has been debated. A major contributor to cutaneous barrier integrity is filaggrin and its breakdown components.[12] Null mutation in *FLG* are associated with increased permeability which would allow allergens to more easily cause sensitization. Such mutations are a major predisposing factor in atopic dermatitis.[13] Their role in allergic contact dermatitis is less clear. Loss of function mutations in *FLG* have been associated with irritant dermatitis[14-17] which in turn would damage barrier function and predispose to allergic

contact dermatitis.[11,18] This is supported by data that shows that loss of functions mutations in *FLG* in association with dermatitis, increases the likelihood of contact sensitization while the presence of *FLG* mutations without dermatitis had no influence[19]. Landeck et al [20] showed no remarkable differences in contact sensitization in patients with *FLG* mutations compared to those having wild type *FLG* and similarly Carlsen et al found no significant association between allergic contact dermatitis and *FLG.*[4] However, it should be noted that the Landeck study used a cohort of occupational hand dermatitis patients, which may explain the lack of association. It has been previously suggested that the size of most haptens is small making them able to penetrate into the epidermis despite the presence of functional filaggrin.[4]

It is difficult to study the contribution of *FLG* loss of function mutation without attempting to eliminate other causes of barrier dysfunction or barrier bypass. Most potent allergens are also potent irritants.[21] Nickel, the most common patch test positive seen,[8] has not been associated with *FLG* mutations,[22] but this could have been due to the frequency of piercings in the population. In this study, we have attempted to avoid confounding factors in evaluating the role of *FLG* null mutations and contact sensitivity. We have shown that in patients referred with a possible contact dermatitis at sites other than the hands there is an association between *FLG* mutations and multiple contact sensitivities. This association was significant in those individuals with a self-reported history of atopic dermatitis.

References

1. Carlsen BC, Andersen KE, Menné T, Johansen JD. Patients with multiple contact allergies: a review. *Contact Dermatitis.* 2008;58:1-8.
2. Tavadia S, Bianchi J, Dawe RS, et al. Allergic contact dermatitis in venous leg ulcer patients. *Contact Dermatitis.* 2003;48:261-265.
3. Saap L, Fahim S, Arsenault E, et al. Contact sensitivity in patients with leg ulcerations: a North American study. *Arch Dermatol.* 2004;140:1241-1246.
4. Carlsen BC, Johansen JD, Menné T, et al. Filaggrin null mutations and association with contact allergy and allergic contact dermatitis: results from a tertiary dermatology clinic. *Contact Dermatitis.* 2010;63:89-95.
5. Lerbaek A, Bisgaard H, Agner T, Ohm Kyvik K, Palmer CN, Menné T. Filaggrin null alleles are not associated with hand eczema or contact allergy. *Br J Dermatol.* 2007;157:1199-1204.
6. Thyssen JP. Atopic dermatitis, filaggrin mutations and irritant contact dermatitis. *Br J Dermatol.* 2013;168:233-234.
7. Molin S, Vollmer S, Weiss EH, Weisenseel P, Ruzicka T, Prinz JC. Deletion of the late cornified envelope genes LCE3B and LCE3C may promote chronic hand eczema with allergic contact dermatitis. *J Investig Allergol Clin Immunol.* 2011;21:472-479.
8. Warshaw EM, Maibach HI, Taylor JS, et al. North American contact dermatitis group patch test results: 2011-2012. *Dermatitis.* 2015;26:49-59.
9. DeKoven JG, Warshaw EM, Belsito DV, et al. North American Contact Dermatitis Group Patch Test Results 2013-2014. *Dermatitis.* 2017;28:33-46.
10. Brown SJ, Asai Y, Cordell HJ, et al. Loss-of-function variants in the filaggrin gene are a significant risk factor for peanut allergy. *J Allergy Clin Immunol.* 2011;127:661-667.
11. Jakasa I, Thyssen JP, Kezic S. The role of skin barrier in occupational contact dermatitis. *Exp Dermatol.* 2018;27:909-914.

12. Koppes SA, Engebretsen KA, Agner T, et al. Current knowledge on biomarkers for contact sensitization and allergic contact dermatitis. *Contact Dermatitis.* 2017;77:1-16.

13. Palmer CN, Irvine AD, Terron-Kwiatkowski A, et al. Common loss-of-function variants of the epidermal barrier protein filaggrin are a major predisposing factor for atopic dermatitis. *Nat Genet.* 2006;38:441-446.

14. de Jongh CM, Khrenova L, Verberk MM, et al. Loss-of-function polymorphisms in the filaggrin gene are associated with an increased susceptibility to chronic irritant contact dermatitis: a case-control study. *Br J Dermatol.* 2008;159:621-627.

15. Landeck L, Visser M, Skudlik C, Brans R, Kezic S, John SM. Clinical course of occupational irritant contact dermatitis of the hands in relation to filaggrin genotype status and atopy. *Br J Dermatol.* 2012;167:1302-1309.

16. Visser MJ, Verberk MM, Campbell LE, et al. Filaggrin loss-of-function mutations and atopic dermatitis as risk factors for hand eczema in apprentice nurses: part II of a prospective cohort study. *Contact Dermatitis.* 2014;70:139-150.

17. Visser MJ, Landeck L, Campbell LE, et al. Impact of atopic dermatitis and loss-of-function mutations in the filaggrin gene on the development of occupational irritant contact dermatitis. *Br J Dermatol.* 2013;168:326-332.

18. Kezic S, Jakasa I. Filaggrin and Skin Barrier Function. *Curr Probl Dermatol.* 2016;49:1-7.

19. Thyssen JP, Linneberg A, Ross-Hansen K, et al. Filaggrin mutations are strongly associated with contact sensitization in individuals with dermatitis. *Contact Dermatitis.* 2013;68:273-276.

20. Landeck L, Visser M, Skudlik C, Brans R, Kezic S, John SM. No remarkable differences in rates of sensitization to common type I and IV allergens between FLG loss-of-function mutation carriers and wild-type subjects. *Contact Dermatitis.* 2014;70:27-34.

21. Kohli N, Nedorost S. Inflamed skin predisposes to sensitization to less potent allergens. *J Am Acad Dermatol.* 2016;75:312-317 e311.

22. Ross-Hansen K, Johansen JD, Voelund A, Menné T, Thyssen JP. The nickel dose-response relationship by filaggrin genotype (FLG). *Contact Dermatitis.* 2014;71:49-53.

Table 1. Demographics and Sites affected stratified by *FLG* genotype.

Demographics	**no *FLG* Mutations (N=137)**	***FLG* Mutations (N=28)**
Female	74% (101)	62% (18)
Average age	54 (SD 15.06)	46 (SD 17.24)
Atopic Dermatitis	31% (43)	43% (12)
Asthma	20% (27)	29% (8)
Hay fever	37% (51)	36% (10)
Food allergies	16% (22)	14% (4)
Family History of Atopic Dermatitis	34% (47)	46% (13)
Family History of Asthma	33% (45)	29% (6)
Family History of Food Allergies	16% (22) *	15% (3) **
Sites affected		
Head and Neck	34% (56)	45% (10)
Widespread	22% (36)	25% (6)
Multiple Sites	13% (21)	13% (3)
Trunk	8% (13)	17% (4)
Feet and Legs	7% (11)	0
Arms	2% (3)	0
Intraoral	1% (2)	4% (1)

* and ** Family history of food allergy not recorded in 24 cases and 8 cases respectively.

SD. Standard Deviation

Table 2. Genotyping results and statistical analysis of filaggrin loss-of-function mutations in patients with polysensitivity (N=165), polysensitivity without a history of atopic dermatitis (N=110) and Canadian controls (N=891).

FLG Genotype	All Patients (N=165)	Controls (N=891)	Patients without Atopic Dermatitis (N=110)
Wild Type	137	793	94
All Mutations	28	98	16
Proportion with FLG Null Mutations	17%	11%	14.5%
X^2	P =0.0299		P = 0.269
Odds Ratio (95% CI)	1.65 (1.05 to 2.61)		1.38 (0.72 to 2.47)

Table 3. Proportion of FLG mutations with increasing number of positive patch tests

	4 Positives N=107	5 Positives N=74	6 Positives N=45	7 Positives N=32
No *FLG* Mutations	87	58	35	23
FLG Mutations	20	16	10	9
Percentage of *FLG* Mutations	18.7%	21.6%	22.2%	28.1%

Table 4. Association between *Filaggrin* mutation carrier status and positive contact reaction to the 20 commonest haptens giving positive reaction in this study.

	All Cases (N=165)			
	Wild Type (N=137)	Mutant (N=28)	OR (95% CI)	*P*-Value
Fragrance mix I	53 (38%)	7 (25%)	0.53 (CI 0.21, 1.35)	0.19
Myroxylon pereirae	30 (22%)	9 (32%)	1.66 (CI 0.67, 4.10)	0.27
Fragrance mix II	14 (10%)	4 (14%)	1.52 (CI 0.45, 5.08)	0.50
Colophony	24 (18%)	5 (18%)	0.89 (CI 0.30, 2.64)	0.83
Cinnamic aldehyde	15 (11%)	2 (7%)	0.59 (CI 0.54, 4.22)	0.50
Formaldehyde	34 (25%)	9 (32%)	1.39(CI 0.57, 3.38)	0.47
Quaternium 15	32 (23%)	8 (29%)	1.40 (CI 0.56, 3.51)	0.48
Diazolidinyl urea	11 (8%)	2 (7%)	0.92 (CI 0.19, 4.44)	0.91
Cobalt	24(18%)	9(32%)	2.19(CI 0.88, 5.46)	0.094
Nickel	25 (18%)	5 (18%)	1.28 (CI 0.42, 3.94)	0.67
Methylisothiazolinone	15 (11%)	1 (4%)	0.34 (CI 0.042, 2.79)	0.32
Lanolin	23 (17%)	8 (29%)	1.98 (CI 0.74, 5.28)	0.17
Paraphenelenediamine	22 (16%)	4 (14%)	1.09 (CI 0.33, 3.60)	0.89
Carba mix	10 (7%)	4 (14%)	1.97 (CI 0.56, 6.89)	0.94
Iodopropynylbutyl carbamate	13 (9%)	3 (11%)	1.01 (CI 0.26, 3.91)	0.99
Thiuram mix	14 (10%)	2 (7%)	0.69 (CI 0.15, 3.24)	0.63
Propolis	13 (9%)	2 (7%)	0.62 (CI 0.13, 3.00)	0.55
Bacitracin	28 (20%)	3 (11%)	0.44 (CI 0.12, 1.58)	0.21
Neomycin	23 (17%)	5 (18%)	1.27 (CI 0.42, 3.83)	0.68
Chromate	11 (8%)	5 (18%)	2.25 (CI 0.70, 7.22)	0.17